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5451003

4. Title of the invention — VACCINE COMPOSITION

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Vaccine Composition

The present invention relates to a composition which is useful for delivering medicaments and particularly vaccines to mucosal surfaces, for example intranasal formulations. The invention further comprises methods of treating individuals using the composition and methods of preparing the composition.

A prime objective in the field of vaccination is the development of a non-parenteral immunisation regimen, which facilitate induction of comparable levels of systemic immunity to that elicited by conventional sub-cutaneous and intra-muscular injections.

The nasapharyngeal passages and pulmonary regions of the respiratory tract represent potential targets for the systemic delivery of peptidergic drugs and vaccines. The relative ease with which therapeutic agents can be inhaled, or introduced into the nose, make these modes of immunisation attractive in terms of probable patient compliance. Furthermore, respiratory mucosae offer certain morphological, physiological and immunological advantages over other non-parenteral sites in terms of immunisation, particularly against pathogenic entitities which affect or utilise mucosal surfaces as portals of entry. This is because effective vaccination against these pathogens normally requires mucosae to be adequately protected with locally produced antibodies of the secretory IgA (sIgA) isotype. Whilst mucosal surfaces are usually poorly protected with IgA following parenteral administration of vaccines, it is now apparent that successful delivery of antigenic material to immunoresponsive elements in mucosa-associated lymphoid tissue (MALT) can result in vigorous stimulation of the mucosal arm of the immune system. By means of the common mucosal immune system (CMIS) it is feasible that several anatomically disparate mucosal surfaces could be protected through mucosal administration of a vaccine at a single sit . Mucosal

vaccination offers the added advantage that some degree of systemic immunity can be induced in concert with local response due to translocation of antigenic material from sub-epithelial compartments to systemic immunoresponsive tissues such as the spleen.

Despite the logistical and immunological factors which favour non-parenteral immunisation, simple mucosal application of antigenic proteins, for example in the gastrointestinal or respiratory tracts, is usually ineffectual in terms of vaccination. Enzymatic or chemical destruction, combined with poor absorption into sub-epithelial compartments dictate that mucosally administered vaccines usually require some form of adjuvant or delivery vehicle. One approach is to encapsulate antigenic material within microparticulate polymeric carriers, such as poly-DL-lactide (PLA) microspheres (Vaccine 1994, 12, 5-11). Such procedures serve to protect labile vaccines from lumenal degradation and enhance adsorption into mucosal and systemic compartments (J.H. Eldridge et al., Seminars in Hematology, (1993), 30, 16-25). There is good evidence that microencapsulation may also adjuvantise by converting soluble antigenic molecules into particulate species, thus promoting vaccine uptake into antigen presenting cells (APC)(Y. Tabata et al., Adv. Polym. Sci. (1990), 94, 107-141, L. Vidard et al., J. Immunol. (1996), 156, 2809-2818, N. Van Rooijen, Immunol. Today (1990) 11, 436-439) or microfold cells (M-cells) in lymphoid follicles (R.I. Walker et al., Vaccine, 12, 387, 1994, D.T. O'Hagan et al,, Vaccine, 1989, 7, 421-424, P.G. Jenkins et al., J. Drug Targetting, 1995, 3, 79-81).

Although comparatively under-investigated, the intra-nasal (i.n.) route is an attractive one for the mucosal delivery of vaccinal entities. The nasal epithelium is accessible and is less exclusive to high molecular weight molecules.

The thickness of the mucus blanket covering respiratory epithelium is relatively thin compared to that of other mucosae, for example the gut where it is in the region of 500 times thicker. Substantially reduced concentrations of proteolytic enzymes and extremes of pH exist in the respiratory tract compared with the gastrointestinal tract.

Furthermore, it is now delineated that nasal associated lymphoids tissues (NALT) have a lymphoepithelium which, like that in the intestinal mucosa, contain M-cells for selective antigen uptake (P. Brandenburg, Immunology of the Lung and Upper Respiratory Tract, (ed. Bienenstoçk J.) McGraw-Hill, New York, 1984, 28-95). Hence NALT plays an analogous role to other MALT, such as the gut associated lymphoid tissues (GALT), in terms of antigen surveillance and induction of mucosal and systemic immunological responses.

The applicants have found that particulate formulations which comprises a polycationic carbohydrate, in particular a chitin derivative such as a chitosan, have an enhanced biological effect when administered by way of a mucosal surface.

Administration to mucosal surfaces may be effected by oral application, by pulmonary application, for example by intra-tracheal administration, or particularly by intra-nasal application. In particular, the compositions of the invention are administered by the intra-nasal route.

Thus the invention provides a pharmaceutical composition for administration to mucosal surfaces, which composition comprises particles comprising

(i) a biologically active agent;

(ii) a first material capable of forming particles; and

(iii) a second material which is cationic and polymeric and which enhances the biological effect of the composition.

Suitably the particles comprise microcapsules or microspheres or liposomes.

The first material used in the compositions of the invention is suitable for forming microspheres or liposomes. Liposome production requires the use of lipids and/or surfactant type molecules as is understood in the art.

Preferably however, the composition of the invention comprises a microsphere. In this case the first material comprises a polymer. It may be a low, medium or high molecular weight polymer. Examples of low molecular weight polymers are polymers which have a molecular weight of between 0.1 and 10kDa, more preferably between 1 and 5 kDa and typically about 2-3kDA.

The use of high molecular weight polymers in the encapsulation of a tetanus vaccine for intramuscular administration has been described (Vaccine 1994, 12, 4, 299-306). A formulation of microencapsulated ricin toxoid vaccine which is applied intranasally has also been described (Vaccine 1994, 14, 11 1031). However, in that case, high molecular weight polymer microcapsules (94kDa) were less effective than those prepared from a copolymer of lower molecular weight (72kDa).

The polymeric material used as the first material in the composition of the present invention suitably has a high molecular weight in excess of 94kDa, for example of 100kDa or more.

A particularly suitable polymeric first material for use in the compositions of the invention comprises poly-(L-lactide) or PLA but other high molecular weight polymeric material such as poly(lactic/glycolic acid) PGLA, polycyanacrylates, polyanhydrides or polycaprotactones as are known in the art may be employed.

The said second material used in the compositions of the invention is suitably a polycationic carbohydrate which enhances the biological effect of the composition. Examples of such compounds include chitin derivatives such as chitosans; cationic polypeptides; cationic polyamino acids; and quaternary ammonium compounds; or mixtures thereof.

Suitably the second material is added to the composition in an amount of from 0.1% to 10%w/w.

The compositions may optionally further comprise agents which stabilise emulsions such as polyvinylalcohol.

They will suitably be of an average size of from 0.1μm to 10μm in diameter.

These compositions may be used to deliver a range of biologically active agents including drugs and pharmaceutical chemicals as well as hormones such as insulin.

These compositions have been found to be particularly effective in the administration of biologically active agent which is capable of generating a protective immune response in an animal, particularly a mammal, to which it is administered. Examples of such agents include antigenic polypeptides as well as nucleic acid sequences which may encode these polypeptides and which are known as "naked DNA" vaccines.

As used herein the expression "polypeptide" encompasses proteins or epitopic fragments thereof.

Suitable polypeptides are sub-unit vaccines, such as tetanus toxoid, diptheria toxoid and *Bacillus anthracis* protective antigen (PA).

In one embodiment, the composition of the invention comprises a biologically active agent which is capable of generating a protective immune response against *Yersinia pestis*. The agent is suitably a sub-unit vaccine, for example as described in WO 96/28551. The vaccine described and claimed there comprises a combination of the V antigen of *Y. pestis* or an immunologically active fragment thereof or a variant of these, and the F1 antigen of *Y. pestis* or an immunologically active fragment thereof or a variant of these.

As used herein, the term "fragment" refers to a portion of the basic sequence which includes at least one antigenic determinant. These may be deletion mutants. One or more epitopic region of the sequence may be joined together.

The expression "variant" refers to sequences of nucleic acids which differ from the base sequence from which they are derived in that one or more amino acids within the sequence are substituted for other amino acids. Amino acid substitutions may be regarded as "conservative" where an amino acid is replaced with a different amino acid with broadly similar properties. Non-conservative substitutions are where amino acids are replaced with amino acids of a different type. Broadly speaking, fewer non-conservative substitutions will be possible without altering the biological activity of the polypeptide. Suitably variants will be at least 60% homologous, preferably at least 75% homologous, and more preferably at least 90% homologous to the base sequence. Homology in this instance can be judged for example using the algorithm of Lipman-Pearson, with Ktuple:2, gap penalty:4, Gap Length Penalty:12, standard PAM scoring matrix (Lipman, D.J. and Pearson, W.R., Rapid and Sensitive Protein Similarity Searches, *Science*, 1985, vol. 227, 1435-1441).

Preferably, vaccine compositions will further comprise a conventional adjuvant in order to enhance the immune response to

the biologically active material administered. Suitable adjuvants include pharmaceutically acceptable adjuvants such as Freund's incomplete adjuvant, aluminium compounds and, preferably adjuvants which are known to up-regulate mucosal responses such as CTB, the non-toxic pentameric B subunit of cholera toxin (CT).

They may also comprise other known composition components such as colouring agents and preservatives and in particular cetrimide. These are suitably present in amounts of from 0.1 to 0.7%w/v.

In a particular embodiment, the microspheres or liposomes used in the compositions may further comprise a coating of S-layer proteins, in particular, S-layer proteins derived from a bacteria against which the biologically active agent produces a protective immune response. It has been shown (Sleyr et al., Crystalline bacterial cell surface proteins. Biotechnology Intelligence Unit, 1996, R.G. Landes Company and Academic Press Inc.) that the stability of liposomes can be increased by such coatings. S-layer proteins are found on the surface of most bacteria and form a regular two dimensional array known as an S-layer. Isolated S-layer proteins are able to form entropy driven monomolecular arrays in suspension, and on the surface of structures such as liposomes.

Compositions of the invention are particularly suitable for intranasal application. They may comprise particles such as microcapsules per se which are optionally preserved, for example by lyophilisation, or the microcapsules may be combined with a pharmaceutically acceptable carrier or excipient. Examples of suitable carriers include solid or liquid carriers as is understood in the art.

The invention further provides a method of producing a pharmaceutical composite run or therapeutic vaccine, which

method comprises encapsulating a biologically active agent as described above in a first polymeric material which has a high molecular weight and in particular a molecular weight of 100kDa or more, in the presence of a second polymeric material such as chitosan. The second material may be incorporated within the microcapsule, or at the surface, of preferably is distributed throughout the microcapsule including at the surface.

Methods of forming liposomes are well known in the art. They include dispersion of dehydrated lipid films into aqueous media, emulsion techniques and lyophilisation methods as are well known in the art.

Microcapsules of the invention are suitably prepared using a double emulsion solvent evaporation method. Briefly, the biologically active agent, suitably in a lyophilised state, is suspended in an aqueous solution of the first polymer such as polyvinyl alcohol (PVA) and the second polymer such as chitosan. A solution of the high molecular weight polymer in an organic solvent such as dichloromethane, is added with vigorous mixing. The resultant emulsion is then dropped into a secondary aqueous phase, optionally containing the second polymeric material, with vigorous stirring. After addition, the organic solvent is allowed to evaporate off and the resultant microspheres separated.

Preferred biologically active agents and first and second materials are as described above.

The compositions of the invention will suitably comprise an appropriate dosage unit of the active agent. This will vary depending upon the nature of the active agent being employed, the nature of the patient, the condition being treated and other clinical factors. In general however, the composition of the invention will comprise approximately 2 to 10 wt% of active ingredient.

In microcapsule containing compositions of the invention the amount of first material, in particular the high molecular weight polymer, in the composition will be of the order of 70 to 99wt% of the composition, and suitably from 90 to 99wt% of the polymer components will be the first polymer. The amount of second polymeric material, such as chitosan or a mixture of chitosan with other positively charged molecules, will be of the order of 0.1 to 10 wt % of the composition.

In use, a reasonable dosage for nasal administration would be of the order of 0.05g.

Preferred compositions of the inventions are vaccine compositions. Thus, in a further aspect, the invention provides a method of protecting a mammal against infection, which method comprises administration of a vaccine composition as described above to a mucosal surface, in particular a nasal surface, of a mammal.

The applicants have demonstrated that it is possible to protect experimental animals from inhalation challenge with *Y. pestis* through i.n. administration of a combined sub-unit vaccine. The adjuvantisation of these sub-units is advantageous in enhancing the immune response as is microencapsulation of the sub-units in accordance with the invention. The high molecular weight polymer utilised in the compositions of the invention appears to be particularly well suited to intra-nasal delivery.

The invention will now be particularly described by way of example with reference to the accompanying drawings in which:

Figure 1 illustrates the serum immune response in mice to nasally delivered microencapsulated and free diptheria toxoid with 10 lf units on day 1 and day 67, where the first column represents results with microsphere without chitosan, the second column represents the results of microspheres with chitosan and

the third column shows the results with free diptheria toxoid alone.

Example 1

Microencapsulation of diptheria toxoid

Poly-L-lactide of molecular weight 100kDa (Polysciences Inc. USA) was used in a modification of the double emulsion solvent evaporation method (Y. Ogawa et al., Chem. Pharm. Bull., 36 (1988) 1095-1103). Briefly, 1.5ml of a 0.75% w/v of chitosan solution containing diptheria toxoid was vigorously mixed with 200mg of 100K PLA polymer dissolved in 5ml of HPLC grade dichloromethane (DCM) using a Silverson homogeniser (Silverson, UK) for 1 minute. The resultant primary emulsion was added, drop by drop, into a secondary aqueous phase (75ml) containing 0.5%w/v chitosan and homogenised using a Silverson homogeniser for 5 minutes. This secondary phase was gently stirred overnight to until the dichloromethane had evaporated. Microspheres were recovered by centrifugation, washed with double distilled water three times and then lyophilised.

Example 2

Immunisation Study

Balb/c female mice (25g, 6-week old) were lightly anaesthetised using an inhaled gaseous mixture of 3% halomethane (RMB Animal Health Ltd., UK) in oxygen ($300cm^3\ min^{-1}$) and nitrous oxide ($100cm^3min^{-1}$) for intranasal dosing procedures. Groups of mice received one of the following treatments:

(1) Microspheres prepared as described in Example 1 but in the absence of chitosan;

(2) Microspheres prepared as described in Example 1; and

(3) free diptheria toxoid solution.

Each were administered in 50µl of PBS using a micropipette. Groups of mice each received 10 lf units on day 1 and day 67 of either microencapsulated or free diptheria toxoid.

Serum immune responses were monitor d. Tail vein blood samples were taken from all animals on days 14, 28, 95 and 151 of the experiment. Titration of IgG and IgA antibody isotypes in serum samples was achieved using an ELISA. Briefly, individual serum samples were aliquoted to microtitre plates pre-coated with diptheria toxoid. Binding of serum antibody was detected with peroxidase-labelled secondary antibody to mouse IgG (Sigma A4416) or AgA (Sigma A4789). Antibody titre was estimated as the maximum dilution of the serum giving an absorbance reading greater that the maximum optical density (OD) of titrated naïve serum. From this, mean titres $\pm$ standard deviation (SD) were derived per treatment group.

The results are shown in Figure 1. Throughout the 151 day schedule, mice dosed with microencapsulated antigen in the absence of chitosan (Group 1) maintained statistically elevated serum IgG titres to diptheria toxoid in comparison to animals treated with free vaccine (Group 3) (Figure 1). However, the levels of IgG titres to diptheria toxoid in Group 2 animals was consistently higher still indicated that the presence of chitosan in the microcapsule enhances the immune response to the toxoid.

Claims

1. A pharmaceutical composition for administration to mucosal surfaces, which composition comprises particles comprising:
(i) a biologically active agent;
(ii) a first material capable of forming particles; and
(iii) a second material which is cationic and polymeric and which enhances the biological effect of the composition.

2. A composition according to claim 1 wherein the second material is a polycationic carbohydrate.

3. A composition according to claim 2 wherein the polycationic carbohydrate is a chitin derivative, cationic polypeptide, cationic polyamino acid, a quaternary ammonium compound or a mixture thereof.

4. A composition according to claim 3 when the polycationic carbohydrate is a chitin derivative.

5. A composition according to claim 4 wherein the chitin derivative is chitosan.

6. A composition according to any one of the preceding claims wherein the particle comprise microspheres, microcapsules or liposomes.

7. A composition according to claim 6 wherein the particle comprises a microcapsule.

8. A composition according to claim 7 wherein the first material is a high molecular weight polymer.

9. A composition according to claim 8 wherein the first polymeric material has a molecular weight of 100kDa or more.

10. A composition according to any one of claims 7 to 9 wherein the first material comprises poly-(L-lactide).

11. A composition according to any one of the preceding claims wherein the ratio of the first to the second material is from 99:1 to 9:1 w/w.

12. A composition according to any one of the preceding claims wherein the biologically active agent is capable of generating a protective immune response in an animal to which it is administered.

13. A composition according to claim 12 wherein the biologically active agent is capable of generating a protective immune response against tetanus, diptheria, or *Yersinia pestis*.

14. A composition according to claim 11 wherein the biologically active agent comprises a combination of the V antigen of *Y. pestis* or an immunologically active fragment thereof, and the F1 antigen of *Y. pestis* or an immunologically active fragment thereof.

15. A composition according to any one of the preceding claims which is adapted for intranasal application.

16. A composition according to any one of the preceding claims which further comprises an adjuvant.

17. A composition according to claim 16 wherein the adjuvant is the non-toxic B-subunit of cholera toxin.

18. A composition according to any one of the preceding claims wherein the composition further comprises a preservative.

19. A composition according to claim 18 wherein the preservative comprises cetrimide.

20. A composition according to any one of the preceding claims wherein the particle is coated with a bacterial S-layer protein.

21. A method of producing a pharmaceutical composite, which method comprises encapsulating a biologically active agent in a first material, in the presence of a second material which is cationic and polymeric and which enhances the biological effect of the composition.

22. A method of protecting an animal against infection by a pathogen, which method comprises administration of a composition according to any one of claims 1 to 17 wherein the biologically active material is able to generate a protective immune response against said pathogen, to a mucosal surface of a mammal.

23. A method according to claim 22 wherein the mucosal surface comprises an intranasal surface.

Abstract

A pharmaceutical composition for administration to mucosal surfaces, which composition comprises particles, such as microcapsules of liposomes comprising
(i) a biologically active agent;
(ii) a first material capable of forming microspheres or liposomes; and
(iii) a second material which is cationic and polymeric and which enhances the biological effect of the composition,
said composition being in the form of a microsphere or liposome. Examples of second materials include chitosan.

The composition is particularly useful for the intra-nasal administration of vaccines.

Serum immune response to nasally delivered microencapsulated and free diphtheria toxoid with 10 lf units on day 1 and day 67

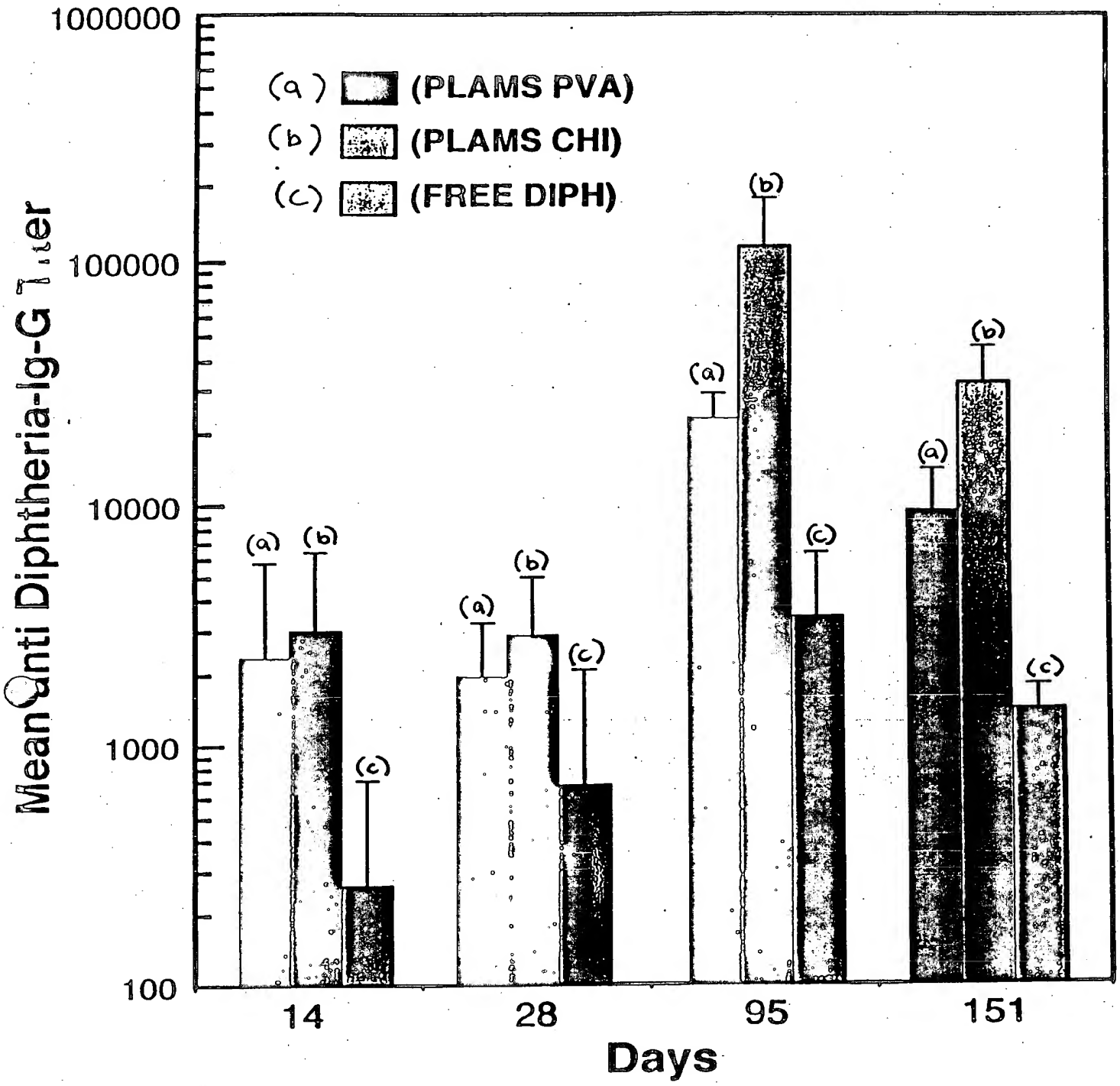

Figure 1

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